REMARKS

This document is filed in reply to the Office Action dated May 25, 2005 ("Office Action"). Applicants have amended claims 1, 3, 5-7, 9, 11-13, 15, 17, 18, 21, 23, 26, 28, 30, and 31 to promote clarity. In particular, Applicants have replaced the term "a *Candida rugosa* lipase" recited in claim 1 or 26, first occurrence, with "a wild-type *Candida rugosa* lipase or a *Candida rugosa* variant." Support for this amendment can be found at page 2, lines 21-23 of the Specification. More specifically, according to this passage of the Specification, "[t]he term '*C. rugosa lipase*' as used herein refers to a pure isozyme, and includes native C. rugosa lipases 1, 2, 3, 4, 5, and 8, as well as their variants." No new matter has been introduced. The amendments should be entered as they raise no new issues that will require further consideration or search and also do not touch the merits of the application within the meaning of 37 C.F.R. § 1.116(b).

Claims 1-35 are pending. Among them, claims 33-35 have been withdrawn for covering a non-elected invention. Upon entry of the proposed amendments, claims 1-32 will be under examination. Reconsideration of this application is requested in view of the following remarks:

Objection under 37 CFR 1.75(c)

In the last office action, the Examiner objected to claims 6, 12, 18, 31, and 32 as being improper dependent form. Each of these claims, dependent from claim 1 or 26, recites a *Candida. rugosa* lipase of SEQ ID NO:4, which is not a wild-type sequence as pointed out in the Specification at page 14, lines 6-8. In the last response dated February 14, 2005, Applicants rectified a typographical error in the Specification.[1] This error might have misled the Examiner to incorrectly interpret the identity of the mutant *Candida. rugosa* lipase of SEQ ID NO:4, as well as the "*Candida. rugosa* lipase" twice recited in claim 1.

The Examiner nonetheless maintained the rejection, stating that these claims fail "to further limit the subject matter of [claim 1] ... which is limited to the wild-type lipase ... [since]

[1] Applicants identified a spelling mistake at page 14, line 7 in the Specification (i.e., "...differs form the wild-type *C. rugosa* lipase 3...") and replaced "form" with "from" to precisely point out that SEQ ID NO:4 is a mutant amino acid sequence which differs from the wild-type sequence.

the *C. rugosa* lipase [recited in claim 1] ... means ... a wild-type lipase." See the Office Action, page 2, lines 3-11 and lines 16-18.

Applicants respectfully traverse. As mentioned above, the term "*Candida. rugosa* lipase" recited in claim 1 "refers to a pure isozyme, and includes native *C. rugosa* lipases 1, 2, 3, 4, 5, and 8, as well as their variants." See the Specification, page 2, lines 21-23; emphasis added. Accordingly, the term at issue refers to both a wild-type *Candida rugosa* lipase and a *Candida rugosa* variant. In other words, contrary to the Examiner's statement, claim 1 is **NOT** "limited to the wild-type lipase." Thus, the Examiner's position is untenable.

In the sole interest of moving this case toward allowance, Applicants have recited "*Candida rugosa* lipase variant" and/or "wild-type *Candida rugosa* lipase" in claims 6, 12, 18, 31, and 32, as well as in independent claims 1 and 26, to promote clarity.

In view of the above amendments and remarks, Applicants request that the objection be withdrawn.

Rejection and Objection under 35 U. S. C. §103(a)

The Examiner maintained the rejection of claims 1-5, 7-11, 13-17, 19-24, and 26-30 as being obvious over Brocca *et al.* or WO 99/14338 ("WO '388") in view of Lotti *et al.* ("Lotti") and Ge *et al.* ("Ge"). See the Office Action, page 2, lines 30-34. Since Brocca *et al.* and WO '388 disclose essentially identical subject matter, Applicants use the term "Brocca" below when referring to both references.

The rejected claims are drawn to (i) a nucleic acid containing a mutant *Candida. rugosa* lipase (CRL) coding sequence, which includes at least 12 universal serine codons substituted for the CTG codons in the wild-type and is at least 80% identical to a wild-type CRL gene; (ii) a microorganism containing the nucleic acid; or (iii) a method of preparing the nucleic acid.

In the last response, Applicants pointed out that (i) Lotti, which teaches molecular cloning and characterization of three CRL genes without mentioning replacement of CTG codons at all, and Ge, which teaches a site-directed mutagenesis procedure to simultaneously introduce multiple mutations, are irrelevant to the rejected claims; and (ii) Brocca, which

describes an unsuccessful effort in making a functional construct containing more than 8 universal serine codons substituted for CTG codons in the wild-type CRL, teaches away from the claims at issue. These references, alone or combined, therefore do not render the claims obvious.

However, the Examiner countered that Applicants' arguments "were attacking references individually." See the Office Action, page 3, lines 7-9. Applicants disagree. Indeed, in the last response, after discussing each of the references in detail (page 24, line 16 to page 26, line 13), Applicants pointed out that they together do not suggest the claims (page 26, lines 13-16, 26, and 27). The very detailed discussions of the individual references might have attributed to the Examiner's misunderstanding of Applicants' arguments.

The Examiner further countered that "Ge *et al.* provide an important teaching of simultaneously mutating several codons in the same time, which is the state of the art at the time of invention. The examiner has taken the position that any nucleic acid sequence thought by one of ordinary skill in the art can be made or modified by chemical or biological means including the methods disclosed by Ge *et al.* The ordinary skill in the art would have had great confidence in his ability and expectation of success to made said nucleic acid regardless of the difficulties mentioned by Brocca *et al.*" See the Office Action, page 3, lines 11-16.

Applicants respectfully traverse and first discuss independent claim 1, which is drawn to the above-mentioned nucleic acid.

According to MPEP 2143, "[t]o establish a prima facie case of obviousness, ... [i] there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. ... [and ii] there must be a reasonable expectation of success." As pointed out in the last response, the four references cited by the Examiner provide neither the required suggestion/motivation nor the required reasonable expectation of success for using the site-directed mutagenesis method taught in Ge in the context of Brocca to generate a construct encoding a *Candida rugosa* lipase.

Here, Applicants note that Ge was published in January 1997 and that Brocca *et al.* was received for publication in December 1997, and WO '388 was filed in September 1997. In other

words, Ge was the state of the art at the time the experiments described in Brocca were conducted, i.e., "in the knowledge generally available to one of ordinary skill in the art." However, using site-directed mutagenesis, Brocca *et al.* failed to make a construct that contains more than 8 universal serine codons substituted for CTG codons and produces a functional lipase. See Brocca *et al.*, page 1416, left column, paragraph 5. WO '388 also teaches that "[i]t did not seem likely the mutagenesis of further serine codons would have an effect as the most relevant candidates vital to activity had already been mutagenised." See page 4, lines 9-11. Furthermore, WO '388 explicitly teaches that "the task of undertaking such large scale mutagenesis is tremendous i.e.[,] an awful lot of effort with very little expectation of success." See the same page, lines 12 and 13; emphasis added.

Given the fact that (i) site-directed mutagenesis taught by Ge was in the knowledge generally available to one of ordinary skill in the art, including Brocca *et al.*; and (ii) Brocca teaches little expectation of success for using site-directed mutagenesis to generate a functional CRL coding sequence having multiple substituted CTG-codons, one skilled in the art would not combine the cited references in the manner alleged by the Examiner. In other words, contrary to the Examiner's statement, one skilled in the art would **not** "have had great confidence in his ability" or any "expectation of success to made said nucleic acid [in view] of the difficulties mentioned by Brocca et al." and also in view of Ge et al.

Lotti teaches molecular cloning and characterization of three CRL genes without mentioning expression of a functional CRL protein. See page 45, Summary. In other words, Lotti does not teach or suggest replacement of CTG codons at all. It therefore does not rectify the above-discussed deficiency of Brocca and Ge et al.

For the reasons set forth above, the Examiner has failed to establish a *prima facie* case of obviousness. Even if a *prima facie* case of obviousness were established, which the Applicants do not agree, it can be successfully rebutted by a showing of an unexpected property of the nucleic acids of claim 1 as compared with the closest prior art nucleic acids, i.e., the nucleic acids taught in Brocca *et al.* or WO '388. More specifically, Brocca *et al.* teaches that

> The mutant genes [or nucleic acids] were cloned into pEMBLyex4, a shuttle expression vector for *S. cerevisiae* cells containing the inducible *UAS GAL* sequence. Recombinant yeast cells grown under inducing conditions, accumulated intracellular **inactive** gene product of lip 1 ... The same result was obtained for all mutants, independently of the number of Ser residues restored.

See page 1416, right column, lines 15-21; emphasis added. That is, lipases encoded by the closest prior art nucleic acids are not functional. In contrast, unexpectedly, nucleic acids of claim 1 encode functional lipases, as evidenced by the data shown in Tables I-V in the Specification.

For the reasons and facts set forth above, Applicants submit that claim 1 is not obvious over Brocca *et al.* or WO '388 in view of Lotti and Ge. Independent claim 19 is drawn to a microorganism comprising the nucleic acid of claim 1. Independent claim 26 is drawn to a method of preparing a mutant CRL gene by conducting PCR amplification to simultaneously introduce at least 12 universal serine codons substituted for CTG codons in the wild-type. For the same reasons and facts set forth above, claims 19 and 26 are not rendered obvious by any combination of Brocca, Lotti, and Ge. Neither are claims 2-5, 7-11, 13-17, 20-24, and 27-30, all of which depend from claim 1, 19, or 26 directly or indirectly.

The Examiner objected to claims 6, 12, 18, and 31 as being dependent upon a rejected base claim. See the Office Action, page 3, lines 34-37. Applicants believe that all of the rejected base claims are now in condition for allowance and this objection should be withdrawn.

CONCLUSION

Applicants submit that the rejections and objections asserted by the Examiner have been overcome, and that claims, as pending, define subject matter that is definite and non-obvious. On this basis, it is submitted that allowance of this application is proper, and early favorable action is solicited.

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Respectfully submitted,

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